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Protective effect of transfection with secretable superoxide dismutase (SOD) (a signal sequence-SOD fusion protein coding cDNA) expression vector on superoxide anion-induced cytotoxicity in vitro.

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For ex vivo gene therapy, superoxide dismutase (SOD) must be secreted into the extracellular space and delivered to damaged cells. Recombinant DNA technique can be used to produce a secretory protein that is fused to a non-secretory protein and a signal peptide of another secretory protein gene. We constructed a secretable SOD eukaryotic expression vector which expresses human SOD cDNA by fusing it to the signal peptide DNA sequence of the human interleukin-2 (IL-2) gene. The ILSOD cDNA constructed by PCR-based gene expression was ligated into the multicloning site of the pRc/CMV plasmid (pRc/CMV-ILSOD). Rat lung epithelial like cells (L2 cells) were transfected with pRc/CMV-ILSOD by lipofection. The extracellular SOD activity of ILSOD-L2 cells (transfected cells with pRc/CMV-ILSOD) was 3 times as high as that of host cells. We used the xanthin (X)/xanthin oxidase (XO) system to produce superoxide anions at the extracellular space. We initially investigated the direct cytotoxicity of superoxide anions upon cells. Host and ILSOD-L2 cells were killed by using X/XO, although the sensitivity of the ILSOD-L2 cells to X/XO induced cytotoxicity was significantly decreased compared with that of host cells. The production of lipid peroxidated substances in the host in the presence of X/XO increased to about twice the control (absence of X/XO) level. However, that of ILSOD-L2 cells did not change in the presence of X/XO. Therefore, ILSOD-L2 cells were resistant to X/XO induced lipid peroxidation. These findings indicated that ILSOD gene transfection protected against direct oxidant stress by X/XO. We then investigated the effect of extracellular SOD secreted from ILSOD-L2 cells on extracellular superoxide anion induced cytotoxicity in normal cells. The conditioned media of host cells had no significant effect upon X/XO induced

cytotoxicity. However, the conditioned media of ILSOD-L2 cells protected against X/XO induced cytotoxicity. Furthermore, the conditioned medium of ILSOD-L2 cells was more effective than that of host cells against the production of lipid peroxidated substances by normal cells under conditions of oxidative stress. These results indicated that non-secretable protein could be delivered to target cells by means of DNA engineering. This strategy could thus provide an ex vivo means of applying gene therapy using non-secretable proteins.

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